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REMARKS

In response to the election requirement, the Applicants withdraw without prejudice claims 3, 10, 19-42, 45, 46 and 48 to 51 and submit the listing of claims enclosed herewith in replacement of all prior versions. Claim 6 is rewritten to reflect correct amendments to the claim.

In this new listing of the claims:

Claims 1, 2, 6 and 7 have been amended;

Claim 8 is original;

Claim 9 has been amended;

Claim 43 has been amended;

Claim 47 is original;

Claims 53 to 57 are new and find proper support in various sections of the specification;

Claims 58 and 59 are new and correspond respectively to claims 12 and 18 presently on file; and

Claims 4, 5, 11 to 18 and 44 presently on file have been withdrawn without prejudice.

As requested by the Examiner in **paragraph 4**, a new and corrected Declaration duly signed by GIDDA SATINDER is enclosed.

In paragraph 5, The Examiner noted that JP 02-092220 listed in the IDS filed by the Applicants has not been provided. Accordingly, a copy of the abstract of this publication in English, and a copy of the publication in Japanese are provided herewith for the Examiner's convenience.

In paragraph 6, the Examiner has rejected claims 6, 14 and 44 presently on file for reading on non-elected inventions. In response to this rejection, the Examiner will note that the new set of claims has been limited to the elected invention.

In paragraph 7, the Examiner has objected to the specification because of the embedded hyperlinks. In response to this rejection the hyperlinks appearing on pages 17 and 18 of the specification have been deleted.

In paragraph 8, The Examiner has rejected claims 6 to 8 and 14 presently on file under 35 U. S. C. 112, second paragraph as being indefinite and failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. In response to this rejection, the new claims now refer to a SEQ ID from the sequence listing.

In paragraphs 9 and 10, the Examiner has rejected claims 1, 2, 4 to 9, 11 to 18, 43, 44 and 47 presently on file under 35 U.S.C. 112 first paragraph as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the art that the inventors, at the time the application was filed, had possession of the claimed invention, and because the specification is only enabling for a method of increasing time to flowering in *Arabidopsis* plants comprising transforming said plants with the *Arabidopsis* AtST2a genomic sequence of SEQ ID NO:1, operably linked to a promoter in antisense orientation, wherein the level of 12- or 11- hydroxyjasmonic acid are increased relative to non-transgenic plants.

In response to this rejection, the Applicants submit a new listing of the claims relating to a method of increasing time to flowering in *Arabidopsis* plants, comprising transforming said plants with the *Arabidopsis* AtST2a genomic sequence of SEQ ID NO:1, operably linked to a promoter in antisense orientation, wherein the level of 12- or 11- hydroxyjasmonic acid are increased relative to non-transgenic plants. Hence, the listing of claims is now fully supported and enabled by the description.

Moreover, the sequence described in the present application contains the motifs that are well known to be present in all soluble sulfotransferases that have been characterized so far. It is possible to find this information by doing a protein blast search at NCBI. More specifically, two domains are highly conserved.

- The first one comprises the sequence YPKSGTTW and is localized at the N-terminal of all sulfotransferases. The lysine residue of this domain has been shown to bind the sulfate donor 3'-phospoadenosine 5'-phosphosulfate (see Marsolais, F and Varin, L. (1995) Identification of amino acid residues critical for catalysis and substrate binding in the flavonol 3-sulfotransferase. *J. Biol. Chem.*, **270**, 30458-30463.)
- The second domain, RKXXGDWKNXFT, is localized closer to the C-terminal extremity.

 The arginin residue of this motif is critical for the binding of the sulfate donor.
- There are also other amino acid residues that have been shown to be absolutely required for activity such as histidine 118 (numbering of the flavonol 3-sulfotransferase) which acts as base catalyst during catalysis.

It is very easy for a person skilled in the art, namely, one having expertise in protein chemistry, to find these conserved domains and to assess the sulfotransferase function to an unknown protein having these motifs.

Another reference that can be used to find the structural characteristics of sulfotransferases is: Marsolais, F. and Varin, L. (1998) Recent developments in the study of the structure-function relationship of flavonol sulfotransferases. *Chem. Biol. Interact.*, 109, 117-122.

The Applicants also submit that knowing the conserved domains present in all soluble sulfotransferases, it is very easy to identify in databases protein sequences having sulfotransferase activity. A FASTA search in SWISSPROT using the string YPKSGTTW will retrieve a large number of sequences that have already been characterized at the biochemical level or that are predicted to encode sulfotransferases.

To search for new sulfotransferase coding sequences, a PCR approach using partially degenerate oligonucleotides targeting the two conserved domains would allow amplification of DNA fragments encompassing a large portion of sulfotransferase coding sequences. The PCR products can then be used to screen libraries to search for full-length clones. Someone skilled in the art <u>can easily</u> perform this molecular approach.

Finally, despite the fact that it is difficult to predict exactly the outcome of plant transformation experiments due to the random insertion of the T-DNA in transgenic plants, the characterization of a number of independent lines allow to find the one(s) expressing the transgene at an adequate level. This is true both for overexpression in sense and underexpression via antisense or RNA interference. Someone skilled in the art, would easily bypass this apparent pitfall of transgenic plant production.

In paragraph 11, the Examiner has rejected claims 1 and 2 presently on file under U. S. C. 102(b) as being anticipated by Krajncic et al. (1995, J. Plant Physiol. 146:754-756). In response to this rejection, claims 1 and 2 have been amended.

Hence new claims 1 and 2 are now directed to a method for modulating or inducing flowering in a plant, comprising modifying in said plant the endogenous level of at least one compound selected from the group consisting of 12-hydroxyjasmonic acid, glucoside of 12-hydroxyjasmonic acid, sulfate ester of 12-hydroxyjasmonic acid, 12-hydroxymethyljasmonic acid, glucoside of 12-hydroxymethyljasmonic acid, sulfate ester of 12-hydroxymethyljasmonic

acid, 11-hydroxyjasmonic acid, glucoside of 11-hydroxyjasmonic acid, sulfate ester of 11-hydroxyjasmonic acid, 11-hydroxymethyljasmonic acid, glucoside of 11-hydroxymethyljasmonic acid, sulfate ester of 11-hydroxymethyljasmonic acid, and mixtures thereof, wherein the endogenous level of at least one compound is modified by modulating the expression of a sulfotransferase encoded by a gene of SEQ ID NO:1.

On the other hand, the scientific article by Krajncic et al. discloses the addition of jasmonic acid solution to the nutrient solution of the experimental plants *Spirodela polyrrhiza* and does not disclose the modulation of the expression of a sulfotransferase encoded by a gene of SEQ ID NO:1.

Moreover, and as described in the application, the novelty of the present invention resides in the fact that the Applicants has demonstrated that it is not jasmonic acid which is responsible for the induction of flowering, but 12-hydroxyjasmonate. The Applicants agree that the treatment of plants with jasmonic acid can affect flowering time but submits that it will also affect other aspects of plant development. The Applicants also agree that a mutation in the jasmonate pathway will ultimately lead to the absence of 12-hydroxyjasmonate and consequently give rise to late flowering plants, but the present invention has the advantage to keep the jasmonate pathway intact. For example, a knock-out mutation of the gene encoding the enzyme Allene Oxide Synthase (AOS) results in plants which are deficient in jasmonic acid (J-H Park et al. 2002) but as stated in the research article, these plants are also defective in wound signal transduction and are more susceptible to pathogen infection.

Similar negative side effects were obtained with plants having a mutation in the genes encoding the enzyme Allene Oxide Cyclase (AOC), 12-oxo-phytodienoic acid reductase (OPR3) fatty acid desaturase (FAD3, 7 and 8) and lipooxygenase 2 (LOX2).

It has been demonstrated that jasmonic acid as well as its precursor 12-oxo-phytodienoic acid (OPDA) regulate the expression of several genes involved in the plant defense response. In addition, it has been demonstrated that the application of jasmonic acid to plants has deleterious effects on growth (such as repression of genes involved in photosynthesis, root growth inhibition and loss of chlorophyll). Similar experiments conducted with 12-hydroxyjasmonate did not give rise to similar negative side effects. This can be explained by the fact that 12-hydroxyjasmonate does not induce or repress the same genes when compared with jasmonic acid or OPDA.

To illustrate the difference between the effect mediated by jasmonic acid and 12-hydroxyjasmonate on gene expression, selected results from an mRNA profiling experiment performed with the *A. thaliana* Affymetrix DNA chips are presented in Table 1, 2 and 3. It is important to note that only selected genes are presented and that these results have not been published. The Affymetrix DNA chips comprise more that 22,000 entries and a large number of genes are clustering with the ones presented in the three Tables.

The results show clearly that jasmonic acid and 12-hydroxyjasmonate have different effects on gene expression. For example, 12-hydroxyjasmonate does not repress the expression of genes involved in photosynthesis (Table 1). Furthermore, 12-hydroxyjasmonate does not induce the expression of THI2.1 a marker gene in the plant defense response (Table 2) A similar result with *THI 2.1* was presented in the publication by Gidda. S *et al.* (2003) *J. Biol. Chem.* 278, 17895-17900.

The microarray results clearly show that 12-hydroxyjasmonate induces gene expression in *A. thaliana* and that this induction is independent of the jasmonic acid induction pathway (Table 3).

To summarize, the advantages of modulating the endogenous levels of 12-hydroxyjasmonate by sulfonation over knocking down the synthesis of jasmonic acid to control flowering time are:

- The absence of negative side effects on growth.
- The absence of negative side effects on the defense response.

Hence, the Applicants submit that new claims 1 and 2 are new over the cited prior art and the Examiner is kindly requested to reconsider his rejection under U. S. C. 102(b).

In view of the above arguments and amendments, the Application is believed to be in condition for allowance.

Table 1. Selected genes encoding enzymes involved in photosynthesis.

Accession	Control	methyl jasmonate*	12- hydroxyjasmonate**
At1g44446	4164***	555	3810
At5g01530	42663	29136	45635
At4g27440	19087	4315	21581
At1g29910	48449	27488	53212
At1g19150	9037	6366	9204

^{*} Plants were treated with 50 micromolar of methyl jasmonate for a period of four hours.

Table 2. Selected genes encoding proteins involved in the plant defense response

Accession	Control	methyl jasmonate	12- hydroxyjasmonate
At5g07010	120	9744	223
At3g55970	130	6956	90
At1g63040	8	296	8
At1g72260	104	3487	64
At1g54020	. 71	2278	59
At5g42900	73	1905	69
At3g16330	16	363	16
At3g20810	198	3950	168
At5g37260	56	1105	50
At2g22880	8	148	9
At3g23550	247	4029	250

^{*} Plants were treated with 50 micromolar of methyl jasmonate for a period of four hours.

^{**} Plants were treated with 50 micromolar of 12-hydroxyjasmonate for a period of four hours.

^{***} Numbers represent normalized gene expression values. Three independent biological replicates were used for this study.

^{**} Plants were treated with 50 micromolar of 12-hydroxyjasmonate for a period of four hours.

^{***} Numbers represent normalized gene expression values. Three independent biological replicates were used for this study.

Table 3. Selected genes induced by 12-hydroxyjasmonate

Accession	Control	methyl jasmonate	12- hydroxyjasmonate
At1g30140	3	3	44
At4g04070	4	7	73
At5g21110	4	3	72

^{*} Plants were treated with 50 micromolar of methyl jasmonate for a period of four hours.

Applicants' undersigned attorney may be reached in our Washington, D.C. office by telephone at (202) 625-3500. All correspondence should continue to be directed to our address given below.

Respectfully submitted,

Gilberto M. Villacorta, Рн.D.

Registration No. 34,038

Robert W. Hahl, Ph.D. Registration No. 33,893

Date: May 19, 2005

Attachments: Declaration

Japanese Publication No. 02-092220 Copy of USPTO date-stamped receipt

Patent Administrator KATTEN MUCHIN ROSENMAN LLP 525 West Monroe Street Chicago, Illinois 60661-3963

Fax: (312) 906-1021

^{**} Plants were treated with 50 micromolar of 12-hydroxyjasmonate for a period of four hours.

^{***} Numbers represent normalized gene expression values. Three independent biological replicates were used for this study.



DECLARATION FOR PATENT APPLICATION

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and, insofar as the in the manner pro	e subject matter of each of wided by the first paragrap	e 38, United States Code, § 120 of any the claims of this application is not deal in of Title 38, United Struces Code § 112 tode of Federal Regulations, § 1.68 white i intermational filing data of this applicat	, I (we reaknowledge on occurred betwee	e the duty to disc	ciose
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POWER OF ATTORNEY: I (we) hereby appoint the attorneys associated with the for puting customer number, to prosecute this application and transact all business in the Peterla and Trademark Office conner and transact all business in the Peterla and Trademark Office conner and therewith.

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PATENT ABSTRACTS OF JAPAN

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29.09.1988

(71)Applicant: JAPAN TOBACCO INC

(72)Inventor: TAZAKI HIROYUKI

TSUJINO YASUKO MATSUKI TOMOKO KODA YASUNORI YOSHIHARA TERUHIKO

(54) POTATO TUBER FORMING AND INDUCING AGENT AND METHOD FOR FORMING AND INDUCING POTATO TUBER

(57) Abstract:

PURPOSE: To surely form and induce large amounts of potato tuber by adding ascorbic acid and jasmonic acid compounds to a culture medium. CONSTITUTION: A stem fragment containing a terminal bud or nod reared by shoot tip culture or rooting transfer method of potato plant is reared in tissue culture medium (e.g. Linsmaier & Skoog) for about 4 weeks to provide an aseptic shoot. 10-5000ppm ascorbic acid and 0.3-12ppm jasmonic acid compound expressed by formula I or formula II (R1 and R2 are H or 1-10C alkyl; R2 is H, OH, O-D-glucopyranose) and as necessary 0.5-10ppm cytokinins compound (e.g., kinetin) used as a potato tuber-forming and inducing agent are added to a culture medium containing the above-mentioned aseptic shoot and the shoot is cultured for 2-4 weeks to form potato tuber at the nod of aseptic shoot.

LEGAL STATUS

[Date of request for examination]

[Date of sending the examiner's decision of rejection]

[Kind of final disposal of application other than the examiner's decision of rejection or application converted registration]

[Date of final disposal for application]

[Patent number]

[Date of registration]
[Number of appeal against examiner's decision of rejection]
[Date of requesting appeal against examiner's decision of rejection]
[Date of extinction of right]

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69日本国特許庁(JP)

OD 特許出頭公開

平2-92220 ◎公開特許公輟(A)

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●公開 平成2年(1920)4月3日

A 01 H 4/00 A 01 N 37/38

C 12 N 5/00 審査請求 朱請求 請求項の数 5

FX (金4頁)

馬鈴客境医形成誘導剤及び同形成誘導方法 40発明の名称 **金村 類 昭63-242432**

說問記号

委出 夏 昭63(1998) 9月29日

神奈川県横浜市県区梅が丘8-2・日本たばこ産業株式会 期 老 ⊞ 仓类 社権的開発研究所候浜センター内 神奈川県被浜市緑区梅が丘8-2 日本たばこ産業株式会 分子 辻 野 社植物開発研究所養浜センター内 神奈川県鉄浜市線区梅が丘6-2 日本たばこ産業株式会 松木 知 0米 社植物開発研究所徴終センター内 北海波札幌市自石区もみじ台西7丁目4番4号 哥 轰 81 盎 仍完 112 審 北极道机能市盘平区西隔四条14丁目 4 春45号 會 質 庭 彦 君 **②**秀 聑

の出頭 人 日本たばこ庭登儀式会 社

東京都港区虎ノ門2丁目2番1号

最終質に続く

項結合

具詮製児蓋粉鼠器審削及び同形鼠器等方法

- アスコルピン酸とジャスモン酸剤化合 後と京方協議分として含なすることを特徴でする
- ジャスモン酸键化合物が12-6-0 - Dーグルコピラノシロキシージャスモン酸、メ チルジャスモン強、ジャスモン酸又は6ーヒドロ ージャスモン酸である請求項1の系給業快签 B4 62 32 NF 37 .
- (3) サイトカイニン酸化合物をも有効状分 として含有する湖京項1又は3の馬鈴羽牧器群成
- サイトカイニン酸化合物がカイネサン である訪水煮るの原給製造業遊成器群群。
- 血族培養精助中に結束領1、2、3又 はもの風拾霜塊監球成體再費を抵加することを修 改とする異常響性霊形点語等方法。

3、発明の詳細な説明

(裁数上の利用分野)

本務明は、無給緊攻器形式語歌和及び同形式語 切方近に関する。 体に、 五銭塩塩万法を用いて馬 免疫処弦形は疑惑する際に有用な馬鈴心児監察点 齢資剤及び国発療験選方法に護する。

く従来の技術と

近郊、鶏舞襲の麒麟培森によって得られる弦器 ※以指数の熱鍵的気温清洁労供に用いることが注 なおれている。この方説においては、馬鈴蘭姫街 を組織培養して、地震を形成譲渡する点にポイン

地球を形成維持するのに関する無供換数貨物の 组成が、「國君學会昭和62年獻本大会は1年2 8月、227页《张表音、秋田 坎、高山耳镇》 において、死に提案されている。

同刊行転では、まず、組務境発塔性であるムラ シザースクーグ (Huraeige-Skoog) 境地のシェー クロース課度を3分に調整した焙炒で遊販焙炙し て、展開シュートを可収(Phase 1)し、次に、

. 特用平2-82220(2)

同場対のシュークロース設置を高模成(8%)に 関勢した増加で超難熔費(Phase 2)して、城路 の形成感を投大させたことが報告されている。

この方法では、Phase まで育成された展出シュートをPhase 2の培地に移植するか、Phase 2の培地に移植するか、Phase 2の塔地になり替えることを必要とし、この際、多大の党力を要する点に組織があった。

(教明が解決しようとする課題)

本義明は、起発技術に見られる上語課題を解決 することもに、一層判効な場合破壊返形成誘導制 及び同利を用いた思熱関雄変形は緊張力性を提供 せんとするものである。

く課品を解決するための予数〉及び(作用) 土和切は、アスコルビン酸とジャスを少敵到化 合物とをお知及分として合有することを特徴とす る思路襲災益形成態複割、アスコルビン酸とジャ スモン難類化合物とサイトカイニン酸化合物とを 材効成分として含有することを特徴とする馬鈴製 機塞形成語溶剤及び前配二額のいずれかを担頼信 銀塩塩に佐加することを特徴とする馬鈴薯残盗

モン酸、メチルジャスモン酸、ジャスモン使又は 8-ヒドロサシージャスモン酸である。

本塾頃に用いわれるサイトカイニン類化合物とは、カイネテン。セニルア モノブリン、フェニルア モノブリン、ソクロヘキシルアモノブリン、ロクロヘナシークロロベンジルアモノブリン、ローメチルペンジルフェニル要数、モービリジルフェニル要数、モービリジルフェニル要数、モーベンジルフィングソール、Bーイソベンテニルアモノブリン、トランスセフテン、ドランスセファンはシド、トランスセファンとフリポチド、ジヒドロゼアテンなどでコエ

展録線境器を利成制率するためには、まず、新 結果が他の器頂点路数又は発根学校により胃原し た頂莎又は節を含む窓前庁(以下、これを「切片」 という、フを組織経業策雄で的も和限庁成して、 熱西ジュートを得る。次に、無関シュートの始雄 中に、アスコルピン酸180~5000ppm、好ましくは 500~2000ppmとジャスモン酸剤化合物の.3~12ppm、 母成計選力性を受賞とするものである。

本独印に用いられるジャスモン放詞化合物とは、次の一般式工又は且で表される化合物である。

上記ジャスモン鉄質む合物は、野ましくは、 1 2-0-0-0-アルコピラノシロモシージャス

哲ましくは1~5ppeとを絵刻し、さらに2~4割 四巻見すると無償シェートの様に復居が形成語述 されるのである。

同様にして、無数ショートの超級中に、アスコルビンは100~5000ppm、日ましくは500~2000ppm、日ましくは500~2000ppm、ビジャスモン酸で化合物0.3~12ppm、日ましくは1~5ppmとサイトカイニン頭化合物0.5~10ppm、日ましくは1~5ppmを送加し、3 らに 2~4 週間増設すると級位シェートの前に領土が遊皮調査をれるのである。

(実施例)

場合健切片を組織格成する培地として、第1数 に示す組成を有するリンスマイヤーースクーグ(Linanaieraskoog)均均(以下、「ひら活動」と 略称する。)を用いた。

終1款し5箱地超成(mg/1)

Hg504 -7H2 0	370	CeC4 2H2 D	440
KHO;	1,900	BR, NO. I	.650
KH2 POL	170	Fe904 - 7H2 0	27.8
Ma. EOTA	37.3	Hn\$0, -4H26	22.3

特册平2-92220(3)

ZnSC+ 2Ha E	6.4	C4804 - SH2 0	Q.025
CoC12 - 6H2 0	0.025	k1	0.83
н, во,	6.2	Healog . SHa D	0.25
シュークロース	30,000	121/31-0	100
塩酸 ナアミン	0.4		

起鉄焙焼は、煎器2.2cm、高さ15cmの質ビン中に15倍地10mlを入れ、20で油紙明条件でも週間焙焼し、平均路長12cmの無値シュートを育成した。無額シュートを開新して得た別片を、あたに、掲載の条件で増減を繰り返し、供試紙面シュートを必要数算成した。

このようにして何な無菌シュートの質ピン中に、 子の形を表に示す軽点に耐難した水路域者100 川を添加した。なりに、20で選続環条件に置き、 2週間後及びも週間後に検照の形成段を設えた。

(MT 25)

的多数		本格板的超級(1997)	
	アスコルヒーンを飲	シン・スキン酸 競化合物	5147:
		(化合物名:抵加及)	
		128-0-2-826-5/5	
本 位明区 3	10ag -	849-9" + 15: 20 : 58, 549	049
本短明医工	10mg	メデカラ~ったもン数: 22・4/4g	9,49
太阳明区 5	1 Ong	э*+スもン数: 21.0 _K g	0×9
		6-24-048-	
本聲明医 4	1 0 a g	BT+Xを3数: 21.2μg	0 нв
本餐明成 5	tong	メデリン・+スモン酸: 22.4,49	وبر25

数·数 粉件·本头块体系组形((ngyith)

対版区は、ループスコルビン酸、18-8- c ーDーグルコピラノシワキシージャスモン酸、メ テルジャスモン酸、ジャスモン酸、8-ヒドロキ レージャスモン酸及びカイネチンをそれぞれが数 に、同様にして添加した単数使用区逆びにシェー クロース設度を9%に調整したLS培地に、反防 シュートを移域した起来災区とし、銀銭投資条件 は、いずれも同一とした。

その結果を第3页に示す。

係3変強性の形成数

	2 翌周後	4 逻阅读
木羟铂氢 1 :	z. I	3.2
本務明区 2	1.7	2.6
木裁甲区 3	2.0	2.7
本指明医 4 .	2.1	3. 2
本范明区 5	2.0	3.1
华孙使司区		
77106~ンパラ:1、000pp#	0	0.3
12-8-0-0-2-036-5/944-		
31.X456位:3.88ppa	0	O
xf65~+ス₹5歳:2,24ppe	• 0	0 . Z
5~+XE5億:2.10ppa	O.	Œ
4-t1-019-9-1XE2M 12.12ps	n O	0 _
14445:2.5ppa .	0	0.1
经运换区	1.6	2. 3
(1) 1 我伊敦は、いずお	5 1 0 E3	変数の平均

注)1、 お皮数は、いずれ 5 1 0 固皮収の平均 値。

2、本程型区の特施には、いずれもアスコ ルピン献1000ppsを会有するとともに、 本発明区1には、18-8-0-D-グルコピタノシロキシージャスセン酸3.88 ppm、本発明区8には、メチルジャスモン酸2.26ppm、本発明区3には、ジャスモン酸2.10ppm、本発明区4には、6ーヒドロキシージャスモン酸2.12ppm、本発明区5には、11まーターの一Dーダルコピラノシロキシージャスモン酸3.88ppm及びカイネチン2.5ppmをも含有する。

第2次から明らかな違り、本名明以1~5は、いずれら2支びも習問題の改善形成及で、従来決 区を上回り、皆れた境蓋形成態があることを示し た。単純使用以は、いずれも被当をほとんど形成 しなかった。

(単独)

特許由頭人 日本たばに建築株式会社

物間平2-92220(4)

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